

## SORPTION OF ORGANIC PHOSPHORUS COMPOUNDS IN ATLANTIC COASTAL PLAIN SOILS

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Organic phosphorus (P) can comprise a significant amount of the total P in animal wastes, yet there is little information on the potential for organic P to be transferred from soils to watercourses. We examined the adsorption of organic P compounds to soils typical of the southeastern United States, i.e., Blanton Sand (loamy, siliceous, thermic, Grossarenic Paleudult), Cecil sandy clay loam (fine, kaolinitic, thermic, Typic Kanhapludult), and a Belhaven sandy loam (loamy, mixed, dysic, thermic, Terric Medisaprist). The behavior of four organic P compounds was studied: adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), and inositol hexaphosphate (IHP); while  $\text{KH}_2\text{PO}_4$  (ortho-P) was used as an inorganic reference. Laboratory studies were conducted to determine the effects of concentration ( $0\text{--}130\text{ }\mu\text{g P mL}^{-1}$ ), pH ( $4.6\text{--}7.6$ ), and soil properties on P adsorption. All the organic P compounds had greater adsorption than  $\text{KH}_2\text{PO}_4$  on the Blanton and Cecil soils at all concentrations and ranges of pH. In the Belhaven soil, IHP had the greatest sorption followed by  $\text{KH}_2\text{PO}_4$  and the nucleotides (ATP, ADP, and AMP, respectively). Adsorption of organic P was positively correlated with soil organic matter and Fe and Al contents. The greater sorption of some organic P compounds over that of ortho-P suggests that these compounds may pose less of a threat to water quality, although this preferential sorption may increase soluble P in situations where there is displacement of ortho-P by organic P added in manures. (Soil Science 2002;167:652-658)

**Key words:** Adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP), inositol hexaphosphate (IHP),  $\text{KH}_2\text{PO}_4$ .

**O**RGANIC phosphorus (P)-containing compounds account for much of the total P in soils, ranging from < 10% in low organic matter mineral soils to 90% in high organic matter soils (Harrison, 1987). The primary organic P compounds identified in soils include inositol phosphates, nucleic acids, phospholipids, and sugar-based phosphates such as glycerophosphate (Black and Goring, 1953). The inositol phosphates gen-

erally predominate and in some cases account for more than 50% of the total organic P present (Turner et al., 2002). The hexa- and pentaphosphates of inositol are present in greater amounts than the lower esters (mono-, di-, tri-, and tetra-). Nucleic acids comprise approximately 3% of soil organic P (Dalal, 1977), while phospholipids comprise approximately 1% (Anderson and Malcolm, 1974).

Many soils receive organic P additions through the application of animal manures, which may contain between 7 to 29 g P  $\text{kg}^{-1}$  dry weight, depending on the diet and animal species (Barnett, 1994). The proportion of organic P may vary between 10% and 80% of the total P and typically decreases as the manure ages (Gerritse and Zuger, 1977; Peperzak et al., 1959). The main forms of organic P found in animal manures are

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nucleic acids (approximately 25% total P), inositol phosphates (approximately 25% total P), and phospholipids (approximately 1% total P) (Barnett, 1994).

Phosphorus applied as manure may behave differently from inorganic P fertilizer in the soil environment and create enhanced solubility and potential mobility of P through the soil profile (Gerritse, 1977; Iyamuremye et al., 1996a and b; Vetter and Steffens, 1980; Vivekanandan and Fixen, 1990). The forms of organic P that are present in animal manures at the time of application could influence their potential impact on water quality, because the various inorganic and organic compounds behave differently in the soil environment. Therefore, it is important to understand how these organic P components behave in soils in order to predict their potential impact on water quality.

The enhanced sorption of some of the organic P components of manure over that of orthophosphate (ortho-P) can displace ortho-P to solution by competition for binding sites (Anderson et al., 1974). This displacement of ortho-P can potentially lead to off-site P losses through runoff and subsurface transport. Other forms of P in manure are rapidly hydrolyzed, such as glycerophosphates and nucleic acid P (Rolston et al., 1975; Goring and Bartholomew, 1952), and become readily available for plant uptake or are potentially lost as soluble P. Manure characteristics, other than organic P content, may affect the P retention capacity of soils. For example, adding manure can complex Fe and Al by organic ligands, which decreases precipitation of P with these metals. These ligands can also compete for P sorption sites, which increases the concentration of soluble P (Iyamuremye et al., 1996a).

As new feed technologies aimed at P reduction in manures are adopted, the composition of manures is altered, which in turn influences its reactivity in soils. For example, the addition of phytase to improve feed digestibility can result in a decrease in total P in manures by up to 40% (Sims et

al., 1999). This reduction in total P may also be accompanied by a change in the forms of P in the manures. For example, preliminary research has shown that these manures contain greater amounts of inorganic P than manure generated from conventional feeds (Sims et al., 1999). The use of genetically mutated corn (containing low levels of IHP) for feed can also reduce the total P excreted by monogastric animals (Spencer et al., 1998), which may change the P composition in the manure. As feed modifications result in manures with less IHP, a situation may be created with an enhanced potential for undesirable P losses.

The sorption of common organic P compounds found in animal manures [IHP, ATP, ADP, and AMP] was studied in three soils typical of the southeastern United States. Although nucleotides (ATP, ADP, and AMP) tend to mineralize quickly in soils, these were chosen to determine the effects of increasing the number of  $\text{PO}_4$  groups on a chain without modifying the main organic component of the molecule. Experiments were designed to identify selected soil properties that influence sorption.

## MATERIALS AND METHODS

### *Soil Collection and Characterization*

Three soils were selected for use in these experiments to represent regions receiving animal manures in North Carolina (Table 1). Samples were collected from the Ap horizon (0–20 cm) on three North Carolina farms. The samples were collected from a Blanton sand (loamy, siliceous, thermic, Grossarenic Paleudults), Cecil sandy clay loam (fine, kaolinitic, thermic, Typic Kanhapludult), and Belhaven sandy loam (loamy, mixed, dysic, thermic, Terric Medisaprists). After sampling, the soil was air dried and sieved (< 2 mm). Soil pH (1:1 soil-to-solution ratio), texture (hydrometer method; Gee and Bauder, 1986) and organic matter content (loss by ignition; Nelson and Sommers, 1996) were measured. Oxalate-extractable Fe and Al were determined by the

TABLE 1  
Selected chemical and physical properties of the soils used in the adsorption experiments

Soil Series	Soil physical and chemical properties*						
	Sand	Silt	Clay	OM	pH	Al <sub>ox</sub>	Fe <sub>ox</sub>
		%		g kg <sup>-1</sup>		mmol kg <sup>-1</sup>	
Blanton sand	90	4	6	13	5.4	22	6
Cecil sandy clay loam	62	14	24	48	6.4	44	25
Belhaven sandy loam	52	29	19	111	5.7	391	85

\*Al<sub>ox</sub> and Fe<sub>ox</sub> indicate oxalate-extractable Al and Fe; Jackson et al., 1986.

method of Jackson et al. (1986), with detection by ICP-OES.

#### Adsorption Isotherm Study

Four organic P compounds (IHP, ATP, ADP, and AMP) and one inorganic compound ( $\text{KH}_2\text{PO}_4$ ) were examined in these studies. All adsorption experiments were carried out in duplicate using a soil-to-solution ratio of 1:20. Solutions containing the P compounds were prepared in  $15 \text{ mmol L}^{-1}$  KCl with either constant P concentration (ranging from 0–130  $\text{mg P L}^{-1}$ ) with varying pH (from approximately 4–7.5) or varying concentrations of organic P compounds at a solution pH of approximately 6. The pH was adjusted with 0.1 M NaOH, and pH measurements were taken at the end of equilibration period. The soils and organic P compounds were equilibrated for either 3 or 24 h at 22 °C on a reciprocating shaker and then immediately filtered through a 0.45- $\mu\text{m}$  membrane. The solutions containing IHP or  $\text{KH}_2\text{PO}_4$  were shaken for either 3 or 24 h, while the solutions containing ATP, ADP, and AMP were only shaken for 3 h to minimize potential hydrolysis.

Adsorption isotherms with the nucleic acid derivatives were initially performed using the same 24-h reaction period used with IHP and  $\text{KH}_2\text{PO}_4$ . However, there was measurable breakdown of the ATP into its degradation products of ADP, AMP, and adenosine (data not shown). Since there was considerable ATP degradation during a 24-h period, the extent of ATP sorption could not be determined as loss from solution, as it was not possible to distinguish between sorption and degradation. Presence of measurable metabolites (ADP, AMP, and adenosine) was evidence to support the degradation of ATP, although their concentrations do not entirely account for the decrease in ATP concentrations. Because of this rapid degradation, the shaking time used in the subsequent adsorption isotherm experiments was decreased to 3 h, as used by McKercher and Anderson (1989). During this time, only minor amounts (< 1%) of ATP degradation products were detected.

Organic P compounds were separated using reverse-phase ion-pairing chromatography with detection by a photo diode array detector (nucleotides) or refractive index detector (IHP) (Leytem and Mikkelsen, 2003). Orthophosphate solutions were analyzed by the molybdate blue method of Murphy and Riley (1962). Adsorption isotherms were then constructed for these compounds on each soil type by calculating the ad-

sorbed concentrations at pH 6 for each of the six initial concentrations.

Sorption maxima were calculated using Langmuir isotherms as follows:

$$K_d = bK - Kq_i \quad (1)$$

where  $K_d$  is the distribution coefficient ( $K_d = q_i/c_i$ ); a graph of  $K_d$  against  $q_i$  is a straight line with slope equal to  $-K$  and an x intercept equal to  $b$  (maximum sorption). Statistical analysis was performed on the sorption maxima using Duncan's multiple range test (DMRT).

## RESULTS AND DISCUSSION

### Sorption of IHP and $\text{KH}_2\text{PO}_4$

There were significant positive correlations between pH and sorption of IHP in the Cecil and Belhaven soils (Fig. 1), but there was no correlation between pH and sorption on the Blanton sand (data not shown). These results are contrary to those previously reported by Anderson et al. (1974), who reported decreasing adsorption of IHP with increasing pH over the range 3 to 7. Sorption of  $\text{KH}_2\text{PO}_4$  was not significantly correlated with changes in pH on any of the three soils tested (data not shown).

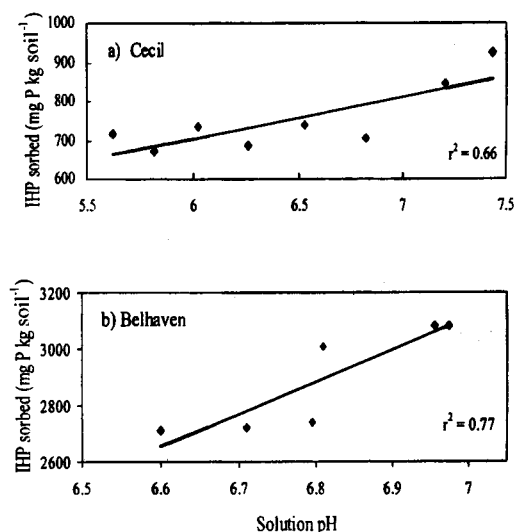


Fig. 1. The effect of pH on the sorption of inositol hexaphosphate on a) Cecil sandy clay loam, and b) Belhaven sandy loam. Solutions were shaken for 24 h at an initial concentration of 105  $\text{mg P L}^{-1}$  and 154  $\text{mg P L}^{-1}$  on the Cecil and Belhaven soils, respectively ( $P = 0.05$ ;  $df = 6$  and 3 for a and b, respectively).

Sorption of IHP and  $\text{KH}_2\text{PO}_4$  increased at higher concentrations, eventually reaching an equilibrium concentration where no more sorption occurred. These results are contrary to those found by Anderson et al. (1974), who reported that as IHP was added to solution, sorption first increased to a maximum point after which it desorbed back into solution. The soils used in the experiments by Anderson et al. (1974) had the same pH range, but with higher added P concentrations than the soils used in this study. The L-type sorption isotherms found with IHP and  $\text{KH}_2\text{PO}_4$  on these soils follow the trends reported by McKercher and Anderson (1989) on neutral and basic soils, where sorption consistently increased with increasing concentration.

The sorption maxima of IHP and  $\text{KH}_2\text{PO}_4$  was greatest in soils having the greatest oxalate-extractable  $[\text{Fe} + \text{Al}]$ , and organic matter, following the order Belhaven > Cecil > Blanton ( $\alpha = 0.05$ ;  $df = 3$ ). The Blanton sand, having little extractable  $[\text{Fe} + \text{Al}]$  or organic matter, showed little retention of either P compound, but as extractable  $[\text{Fe} + \text{Al}]$  content increased (Cecil soil), there was an increase in the amount of each compound sorbed. An increase in extractable  $[\text{Fe} + \text{Al}]$  and organic matter content (Belhaven soil) resulted in additional sorption of each compound. McKercher and Anderson (1989) also found that soils with elevated organic matter content ( $\sim 75 \text{ g kg}^{-1}$ ) sorbed large amounts of IHP and  $\text{KH}_2\text{PO}_4$  ( $2500 \text{ mg P kg}^{-1}$  soil for IHP and  $400 \text{ mg P kg}^{-1}$  soil for  $\text{KH}_2\text{PO}_4$ ).

The enhanced sorption of  $\text{KH}_2\text{PO}_4$  on the Belhaven soil may be due to the elevated concentration of oxalate-extractable  $[\text{Al} + \text{Fe}]$  creating conditions favorable for formation of insoluble Al and Fe phosphate compounds. Therefore, inorganic P removed from solution was probably a result of sorbed P both on the soil mineral surfaces as well as Fe and Al complexes (Freese et al., 1992; Van der Zee and Van Riemsdijk, 1986).

Freese et al. (1992) found that P sorption was linearly related to the sum of oxalate-extractable  $[\text{Fe} + \text{Al}]$ . For a given reaction time ( $t$ ) and P concentration ( $c$ ), the amount of P that has reacted with the soil could be described by:

$$F_r(c, t) = \alpha_r(c, t)[\text{Fe}_{\text{ox}} + \text{Al}_{\text{ox}}] \quad (2)$$

where  $F_r$  is the measured P sorption,  $\alpha_r$  is the molar ratio of P sorption, and  $[\text{Fe}_{\text{ox}} + \text{Al}_{\text{ox}}]$  is the sum of oxalate-extractable  $[\text{Fe} + \text{Al}]$  expressed in  $\text{mmol kg}^{-1}$ . Using this approach, Freese et al. (1992) calculated an  $\alpha$  value of 0.14 for a 4-day

equilibration period and 0.19 for a 40-day period. When the  $\alpha = 0.14$  value was used to predict P sorption from  $\text{KH}_2\text{PO}_4$  on the Blanton, Cecil, and Belhaven soils, the Belhaven soil was predicted to adsorb 17 times the amount of P as the Blanton soil and approximately 7 times as much as the Cecil soil. Plots of predicted  $\text{KH}_2\text{PO}_4$  sorbed vs. actual  $\text{KH}_2\text{PO}_4$  sorbed showed a good model fit ( $r^2 > 0.99$ ). This fit supports oxalate-extractable  $\text{Fe} + \text{Al}$  as the primary adsorptive medium for inorganic P in these soils. Anderson et al. (1974) also noted a positive correlation between the sorption of IHP and oxalate-extractable  $[\text{Fe} + \text{Al}]$  concentrations.

#### The Sorption of ATP, ADP, and AMP

There was a significant positive pH effect on sorption of ATP for all three soils (Fig. 2). However, when solution pH was raised above pH 6.5, there was a consistent decrease in ATP sorption

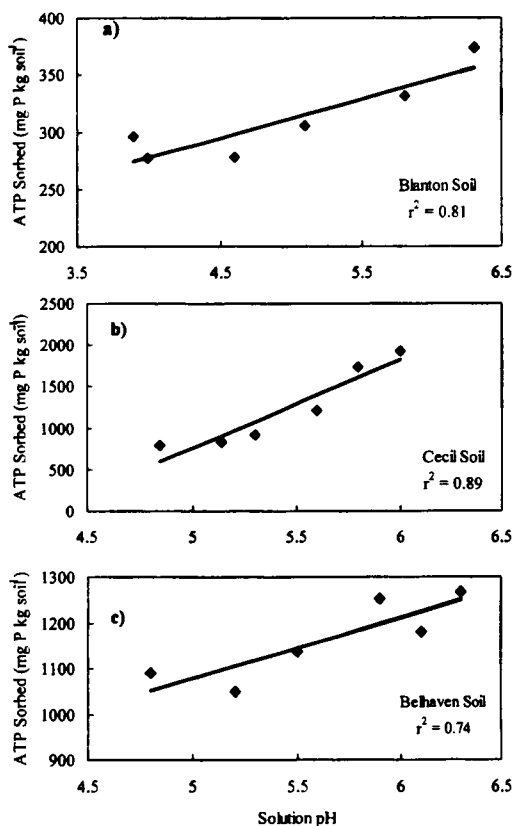


Fig. 2. The effect of pH on sorption of adenosine 5'-triphosphate on a) Blanton sand, b) Cecil sandy clay loam, and c) Belhaven sandy loam. Solutions were shaken for 3 h at an initial concentration of  $130 \text{ mg P L}^{-1}$  ( $P = 0.05$ ;  $df = 4$ ).

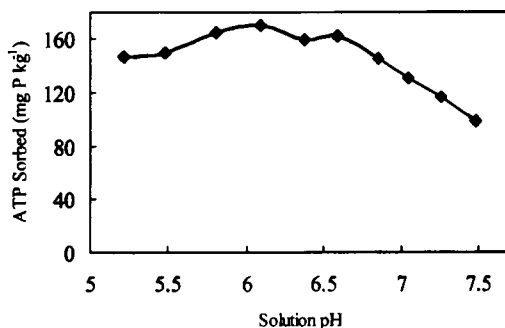


Fig. 3. The sorption of adenosine 5'-triphosphate with increasing pH on Blanton sand. Solutions were shaken for 3 h at an initial concentration of  $169 \text{ mg P L}^{-1}$ .

on the Blanton soil (Fig. 3). This trend is frequently observed with sorption of anions over a wide range in pH. Anion adsorption initially increases with pH and reaches a maximum close to the  $\text{pK}_a$  for anions of monoprotic conjugate acids, while slope breaks are observed at  $\text{pK}_a$  values for anions of polyprotic conjugate acids (Sparks, 1995). In this case, the slope break occurs at pH 6.5, the  $\text{pK}_a$  value for ATP. In contrast, no significant changes in ADP or AMP sorption were observed over the pH range studied (data not shown). The decrease in the number of acidic functional groups on the ADP and AMP compared with ATP may be responsible for the lack of change in sorption with changes in pH.

The sorption maximum of nucleotides by the three soil types followed the trend Belhaven > Cecil > Blanton ( $\alpha = 0.05$ ;  $df = 3$ ). As with IHP and  $\text{KH}_2\text{PO}_4$ , when the oxalate-extractable  $[\text{Fe} + \text{Al}]$  and the organic matter content of the soil increased, there was greater sorption of the nucleotides (Table 1).

#### Comparison of All Compounds on the Three Soils

A comparison was made of the sorption maxima of IHP, ATP, ADP, AMP, and  $\text{KH}_2\text{PO}_4$  on the Blanton, Cecil, and Belhaven soils (isotherms of compounds obtained separately and then compiled (Fig. 4 and Table 2)). The sorption maxima of all of the organic P compounds was greater than  $\text{KH}_2\text{PO}_4$  on both the Cecil and Blanton soils. The sorption maximum of  $\text{KH}_2\text{PO}_4$  was greater than the nucleotides on the Belhaven soil, but less than that of IHP. The general trend seems to follow that as the number of phosphate groups on the organic molecule increases there is an increase in sorption. The difference in sorption between the nucleotides was less pronounced, with

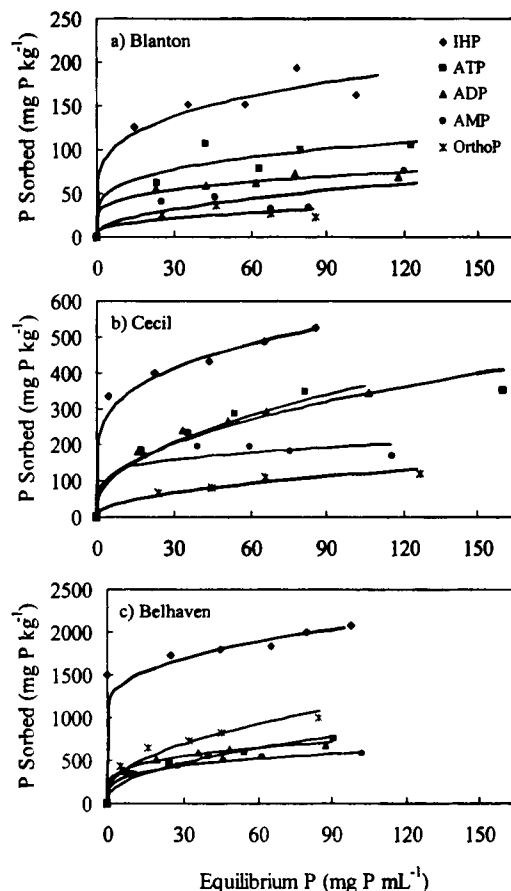


Fig. 4. The sorption of phosphorus compounds on a) Blanton sand, b) Cecil sandy clay loam, and c) Belhaven sandy loam at pH 6. Solutions were shaken for 3 h.

TABLE 2  
Langmuir sorption maxima for phosphorus compounds on three Atlantic Coastal Plain soils

p compounds	Soil series		
	Blanton	Cecil	Belhaven
	mg P kg <sup>-1</sup>		
IHP	213.3 (0.6)*†	575.6 (7.8) <sup>a</sup>	2753.5 (447.6) <sup>a</sup>
ATP	146.3 (19.4) <sup>b</sup>	482.5 (53.0) <sup>b</sup>	837.5 (31.8) <sup>b</sup>
ADP	88.5 (10.6) <sup>c</sup>	379.0 (12.7) <sup>c</sup>	607.3 (49.1) <sup>c</sup>
AMP	93.3 (4.7) <sup>c</sup>	238.3 (0.9) <sup>d</sup>	630.0 (24.0) <sup>c</sup>
$\text{KH}_2\text{PO}_4$	44.0 (2.8) <sup>d</sup>	111.0 (7.1) <sup>c</sup>	1253.5 (55.9) <sup>d</sup>

\*Numbers in parentheses indicate the standard deviation of the mean.

†Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

ATP sorption maxima greater than ADP and AMP on all of the soils, while there was only a significant difference between ADP and AMP sorption on the Cecil soil.

The behavior of the organic P compounds examined in this study has implications for the potential reactivity of P in soils receiving heavy applications of animal manures. In all cases, IHP had greater sorption than the nucleotides as well as  $\text{KH}_2\text{PO}_4$ . Anderson et al. (1974) found that soils either pretreated with IHP or having combinations of IHP and ortho-P in solution had decreased sorption of ortho-P, indicating that there was a preference in sorption of IHP over ortho-P. As IHP can comprise as much as 50% of total P in some manures (Barnett, 1994), the addition of manures can lead to preferential adsorption of IHP and the release of ortho-P into solution. Ortho-P released from manure applications would then be available for plant uptake or potentially lost as soluble P in areas where P concentrations exceed that needed for plant growth. Although the sorption of the nucleotides was greater than that of ortho-P in two soils tested, they are readily mineralized in soils and therefore do not have as great an effect on total P sorption as IHP.

### CONCLUSIONS

Understanding the potential for P transport in areas receiving large applications of animal manures is complicated. There are many factors related to manure composition and the forms of P contained in manures which can affect the potential sorption of P in soils and its potential for off-site transport. More research is needed to fully understand the mechanisms for P retention and transport from soils. As new animal feed strategies are developed and adopted to modify the amount and composition of P in manure, close attention is required to assess how these new management techniques affect manure composition and the solubility of the P forms present.

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